PHENOL BIODEGRADATION OF IMMOBILIZED *BRADYRHIZOBIUM JAPONICUM* CELLS. PART 2

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Abstract

With the development of technologies and the rise of the standard of living worldwide, the generation of wastewater is steadily increasing and at the same time the requirements for their purification are being increased before they are released into the environment. Wastewater treatment methods are diverse, chemical ones which have a significant drawback, are expensive and generate secondary pollutants. These disadvantages are avoided by the use of biological methods, which are very flexible and easy to manage. This article examines the ability of cells from the Bradyrhizobium japonicum 273 strain, successfully immobilized on activated carbon, to oxidize and degrade phenol. Initial pollutant concentrations are (in g dm^{-3}): 0.02, 0.04, 0.06 and 0.08, but they do not have a significant effect on the rate and amount of phenol degradation, which in 240 hours is approximately 10 g dm^{-3}. It is higher by 80% than for free cell degradation rate. In our opinion, the reason is that the activated carbon adsorbs the phenol and gradually releases it in cell-tolerant amounts, i.e. thus avoiding substrate inhibition.

Key words: *bradyrhizobium japonicum, biodegradation, phenol, immobilized cells*

1. INTRODUCTION

A large number of *Rhizobium* microorganisms are able to degrade many types of xenobiotics, phenol in particular. The ability of *Rhizobia* to use carbon sources as aromatic compounds has been studied before [1 – 9].

The degradation of phenol from *Bradyrhizobium japonicum* has not been extensively investigated, but its possibilities for oxidation of other aromatic compounds, which S. Latha and A.Mahadevan [7] have systematized in Table 1, are known.

The results of our laboratory studies on the ability of *Bradyrhizobium japonicum* to break down phenol have been systematized and described in a two-part article. In the first part, we have covered what phenol is, how dangerous it is, how it falls and where it comes from in waste and groundwater, and what its metabolic pathway is. The experimental material presented in the first part reflects the experiments carried out with free cells and in the second one - with immobilized cells on activated carbon. The experiments were conducted with two working media – poor, i.e. mineral one and another one rich in yeast extract and glucose. We investigated the effect of different initial pollutant concentrations on the rate and amount of degraded phenol. The impact of the additional carbon source was also investigated. As a result, we prove the advantage of immobilized cells from the *Bradyrhizobium japonicum* strain to the free ones.

Table 1. Utilization of phenolic compounds.

Strains	**Aromatic compounds**	**References**
Bradyrhizobium Japonicum 273	p-Hydroxybenzoic acid Protocatechuic acid Salicylic acid	[3]
	Catechin Catechol p-Hydroxybenzoic acid Protocatechuic acid Salicylic acid	[6]
	Benzoate Catechol Cinnamate p-Coumarate Ferulate Gallate Gentisate Mandelate p-Hydroxybenzoate Protocatechuate Syringate Vanillate	[9]
	p-Coumarate p-Hydroxybenzoate Protocatechuate Vanillate	[10]
	Catechin Hydroxyquinol Phloroglucinolcarboxylic acid Protocatechuic acid Resorcinol	[12]
	p-Hydroxybenzoate Protocatechuate	[13]

2. MATERIALS AND METHODS

2.1. Strains of bacteria and culture media

The strain *Bradyrhizobium japonicum 273* is obtained from the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures.

The cultural medium consists of (in g dm^{-3}): Na_2HPO_4 .$12H_2O$ - 5.37; $(NH_4)_2SO_4$ - 0.5; K_2HPO_4-0.5; $Mg_2SO_4 \cdot 7H_2O$ - 0.2; NaCl - 0.1; yeast extract - 1; glucose – 1; tap water – 1 dm^{-3}.

2.2. Working media

2.2.1. Poor medium

The poor medium contained (in g dm^{-3}): $Na_2HPO_4.12H_2O$ – 5.37, KH_2PO_4 - 1.36; $(NH_4)_2SO_4$ – 0.5; $MgSO_4.7H_2O$ – 0.2; supplemented with 5 ml per liter trace element solutions (in g dm^{-3}): $CaCl_2$,- 0.53; $FeSO_4.7H_2O$ – 0.2, $ZnSO_4.7H_2O$ – 0.01; H_3BO_3, - 0.01; $CoCl_2.6H_2O$ – 0.01, $MnSO_4.5H_2O$ - 0.0046; $Na_2MoO_4.2H_2O$ – 0.003; $NiCl_2.6H_2O$ – 0.002.

A 5% (wt) phenol solution with initial concentrations of 0.02 to 0.08 g dm^{-3} is the only carbon source in the medium.

2.2.2. Rich medium

The rich medium contains all the elements like the poor one with the addition of two carbon sources; yeast extract - 0.25 g dm^{-3} and glucose – 1 g dm^{-3}. The rich medium contains all the components like the poor one plus yeast extract and glucose.

2.3. Cells immobilization

Choosing a carrier for cell immobilization, we selected granular activated carbon (Fujikasau, Japan, with specific area of 680 m^2/g.). We were guided by the following: first, the microbial cells are easily attached to its surface, second, the toxic pollutants are adsorbed by the carbon. Therefore, their concentration is reduced to fully endurable values for microbial cells, i.e. we avoid the substrate inhibition working at much higher pollutant concentrations [14].

The microbial suspension, which is in the exponential phase of its development, is mixed with washed and dried to constant weight activated carbon. Afterwards the mixture is placed on a shaker for at least 48 hours at temperature 30 ℃ and 200 rpm.

The microbial cells are bound to the surface of the carbon particles by chemical adsorption. A large number of microorganisms can produce exo-polysaccharides in the biofilm layer, which serve as binding agents between carbon particles and microbial cells [14]. The volume of activated carbon with an immobilized cells is 10% by volume of the working medium.

2.4. Analyzes

2.4.1. Concentration of phenol

Phenol is determined photometrically by a standard colorimetric method based on the formation of a red coloured compound by treating the phenol-containing sample with 3.5% 4 amino antipyrine, 20% ammonium persulfate and the presence of buffer (50 g of NH_4Cl in 900 ml of distilled water), with pH loaded with ammonia to 9.3. The sample is centrifuged and then treated with the above mentioned reagents. After 15 minutes the optical density was measured at wavelength λ = 540 nm. The concentration of phenol was calculated by calibration straight line.

2.4.2. Detachment of cells

The detachment of cells from the surface of activated carbon was monitored by measuring biomass during the course of the experiment. Biomass is measured spectrophotometrically at wavelength λ = 610nm.

3. RESULTS AND DISCUSSION

The laboratory experiments were carried out in 250 ml flasks in a rotary shaker at 30 degrees and 100 rpm. Two media were used as described in part I of this article. The experiments were carried at different initial concentrations and supplementing periodically by different amounts of phenol.

The results of the comparison considered the experiments of the poor with rich media at an initial phenolic concentration of 0.02 g dm^{-3} are shown in **Fig. 1**. **Figure 2** presents the results for initial concentration of 0.04 02 g dm^{-3}. The results for initial concentration of 0.06 g dm^{-3} are shown in **Fig.3** and **Fig. 4** shows the results for initial concentration of 0.06 g dm^{-3} . The figures show, that the various initial concentrations as well as the presence of a second carbon source do not significantly affect the oxidation and the amount of degraded phenol, which is approximately 10 g dm^{-3} in 240 hours. The reason is that the carrier for the immobilization – the activated carbon, sorbs both the amounts of the second carbon source (glucose) and the pollutant. As a result, the concentration of phenol is endurable for microorganisms, i.e., substrate inhibition is avoided. Another issue that can be seen from the graphs, is that for 240 hours the amount of separated cells from the carrier that we observe by measuring the optical density of the medium is negligible. That is why one may conclude that the main contribution in phenol biodegradation is assigned to the immobilized bacteria. Another important results is that the total amount of biodegraded phenol is practically independent of its initial concentration. It could be explained by the strong effect of adsorption on the surface of the activated carbon.

In our experiments, the free culture of the strain *Bradyrhizobium japonicum* (Part 1 of this study) for 240 hours degrades an average of 1 g dm^{-3} of phenol compared to the immobilized ones, i.e. approximately 10 g dm^{-3}.

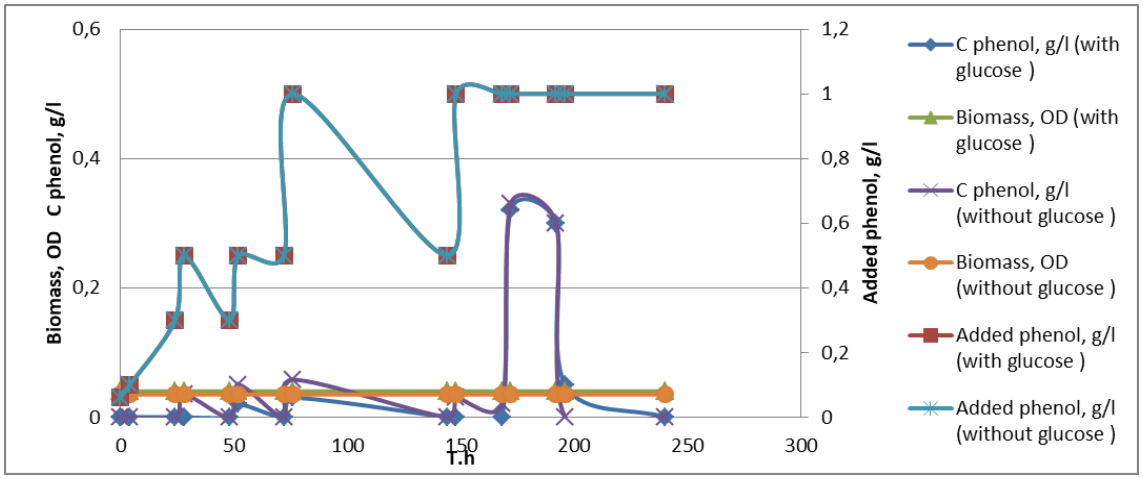

Fig. 1. Comparison of the experimental results of the poor with the rich medium at an initial phenol concentration of 0.02 g / l.

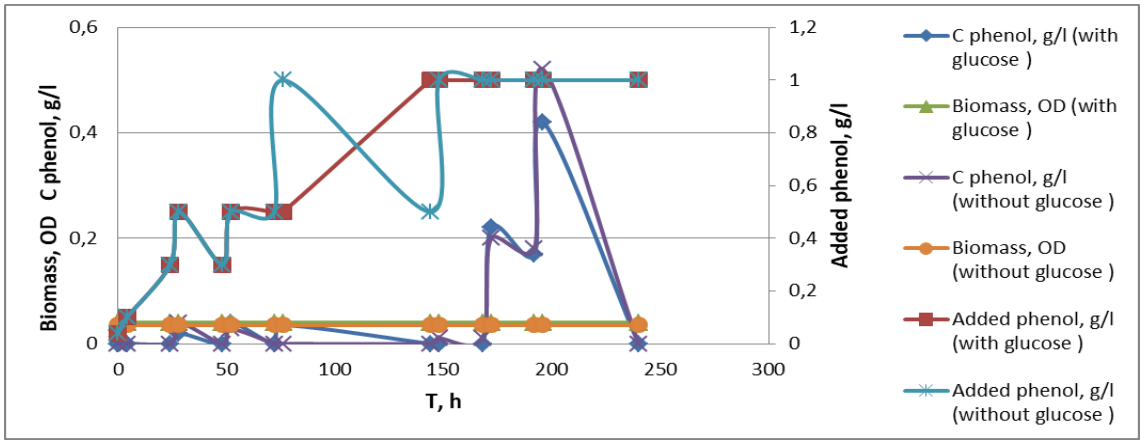

Fig. 2. Comparison of the experimental results of the poor with the rich medium at an initial phenol concentration of 0.04g / l.

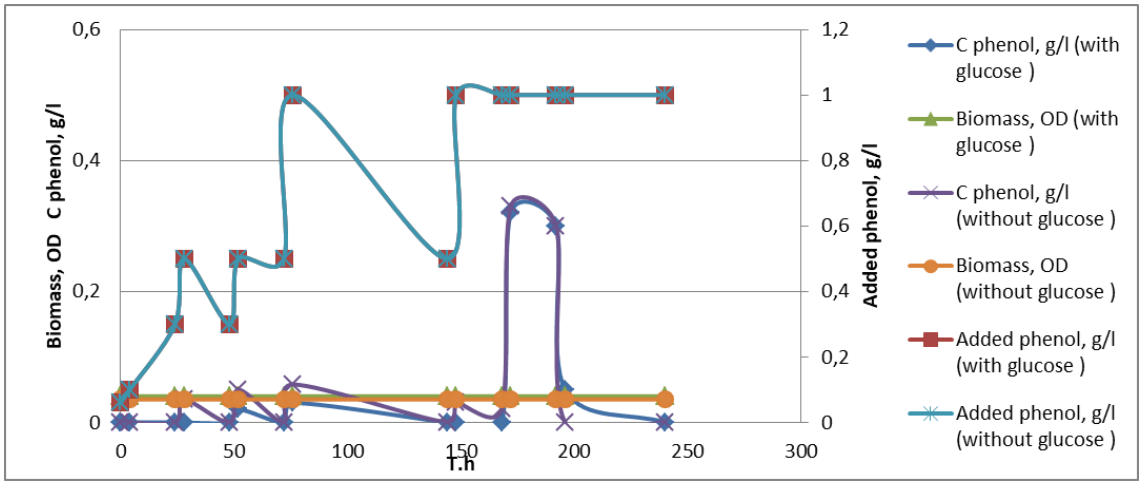

Fig. 3. Comparison of the experimental results of the poor with the rich medium at an initial phenol concentration of 0.06 g / l.

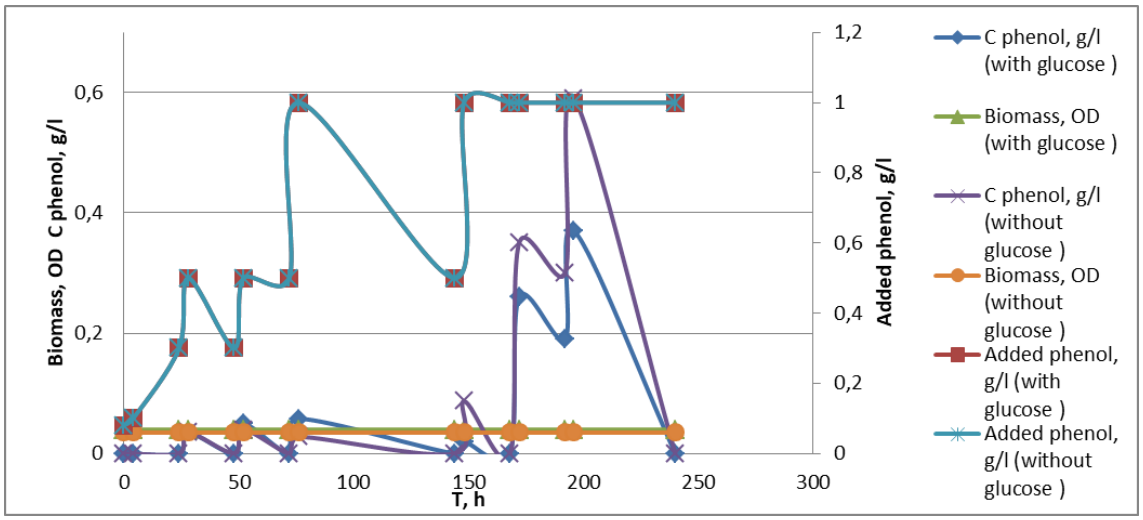

Fig. 4. Comparison of the experimental results of the poor with the rich medium at an initial phenol concentration of 0.08 g / l.

4. CONCLUSION

1. The various initial phenol concentrations (0.02, 0.04, 0.06 and 0.08 g dm^{-3}) do not affect the rate and amount of degraded pollutant for immobilized cells of the *Bradyrhizobium japonicum* strain. The total degraded amount was approximately 10 g dm^{-3} for 240 hours.
2. The presence of a second carbon source does not affect the rate and amount of degraded phenol.
3. During the course of the experiments, the amount of detached cells from the immobilization medium was negligible. That is why the phenol biodegradation is due to the immobilized cells mainly.

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